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Signal Transduction through the Fibronectin Receptor Induces Collagenase and Stromelysin Gene Expression

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Abstract. We have investigated the effects of ligation of the fibronectin receptor (FnR) on gene expression in rabbit synovial fibroblasts. Monoclonal antibodies to the FnR that block initial adhesion of fibroblasts to fibronectin induced the expression of genes encoding the secreted extracellular matrix-degrading metalloproteinases collagenase and stromelysin. That induction was a direct consequence of interaction with the FnR was shown by the accumulation of mRNA for stromelysin and collagenase. Monoclonal antibodies to several other membrane glycoprotein receptors had no effect on metalloproteinase gene expression. Less than 2 h of treatment of the fibroblasts with anti-FnR in solution was sufficient to trigger the change in gene expression, and induction was blocked by dexamethasone. Unlike other inducers of metalloproteinase expression, including phorbol diesters and growth factors, addition of the anti-FnR in solution to cells adherent to serum-derived adhesion proteins or collagen produced no detectable change in cell shape or actin microfilament organization. Inductive effects were potentiated by cross-linking of the ligand. Fab fragments of anti-FnR were ineffective unless cross-linked or immobilized on the substrate. Adhesion of fibro-

blasts to native fibronectin did not induce metalloproteinases. However, adhesion to covalently immobilized peptides containing the arg-gly-asg sequence that were derived from fibronectin, varying in size from hexapeptides up to 120 kD, induced collagenase and stromelysin gene expression. This suggests that degradation products of fibronectin are the natural inductive ligands for the FnR. These data demonstrate that signals leading to changes in gene expression are transduced by the FnR, a member of the integrin family of extracellular matrix receptors. The signaling of changes in gene expression by the FnR is distinct from signaling involving cell shape and actin cytoarchitecture. At least two distinct signals are generated: the binding of fibronectin-derived fragments and adhesion-blocking antibodies to the FnR triggers events different from those triggered by binding of the native fibronectin ligand. Because the genes regulated by this integrin are for enzymes that degrade the extracellular matrix, these results suggest that information transduced by the binding of various ligands to integrins may orchestrate the expression of genes regulating cell behavior in the extracellular environment.

THE interactions of cells with components of the extracellular matrix (ECM)¹, such as fibronectin (Fn), laminin (Ln), tenascin, and collagens of more than 12 types, play an important role in morphogenesis, tissue repair and regeneration, and metastasis (Liotta et al., 1986; Chiquet-Ehrismann et al., 1986; Humphries et al., 1986;

Gehlsen et al., 1988a,b). During development and remodeling, cells in tissues constantly alter their morphology, migration, and adhesion to ECM components. Temporal, spatial, and cell type-specific regulation of the expression of this large variety of ECM molecules and their receptors provides a powerful set of mechanisms for generating the diversity required for the proper orchestration of cell behavior during differentiation, morphogenesis, and tissue remodeling.

The integrin multigene family of transmembrane, heterodimeric adhesion receptors mediates cell attachment to a variety of ECM molecules, including Fn, Ln, collagen types I, IV, and VI, vitronectin (Vn), fibrinogen, and thrombospondin (reviewed by Ruoslahti and Pierschbacher, 1986; Buck and Horwitz, 1987). Antibody and peptide inhibition studies have implicated these receptors in processes as di-

Portions of this work have appeared previously in abstract form (Tremble, P. M., C. Damsky, and Z. Werb. 1988. *J. Cell Biol.* 107:150a).

1. Abbreviations used in this paper: CM, cell-conditioned medium; DME-LH, serum-free DME supplemented with 0.2% lactalbumin hydrolysate; ECM, extracellular matrix; Fn, fibronectin; FnR, fibronectin receptor; LDL-R, low density lipoprotein receptor; Ln, laminin; RSF, rabbit synovial fibroblasts; TIMP, tissue inhibitor of metalloproteinases; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; Vn, vitronectin.

verse as neurite outgrowth (Bozyczko and Horwitz, 1986; Hall et al., 1987; Tomaselli et al., 1987), gastrulation (Boucalt et al., 1984), neural crest cell migration (Thiery et al., 1985; Bronner-Fraser, 1986), trophoblast outgrowth (Damsky et al., 1985b; Richa et al., 1985; Armant et al., 1986; Sutherland et al., 1988), platelet aggregation (Pytela et al., 1986; Phillips et al., 1988), muscle cell attachment to tendons (Bogaert et al., 1987), and tumor cell metastasis (Humphries et al., 1986; Gehlsen et al., 1988a).

In addition to establishing a particular set of contacts with the ECM, cells must be able to modify these contacts in a closely regulated fashion. Mechanisms by which this might be accomplished include regulation of the amounts of ligands and receptors present or alteration of ligand-receptor affinity. Proteolysis of ligands or receptors is likely to be involved in the processes of migration, invasion, and tissue repair (Liotta et al., 1986). Inhibition of proteinases inhibits invasion but not attachment of tumor cells (Mignatti et al., 1986; Schultz et al., 1988). Proteinases are present focally at adhesion sites in some cells, actively modulating their actin cytoskeleton and attachments to the substrate (Beckerle et al., 1987; Pöllänen et al., 1988). There is also a strong correlation between changes in the actin cytoskeleton and the induction of expression of collagenase and stromelysin (Aggeler et al., 1984a,b; Werb et al., 1986), two members of the metalloproteinase gene family (Whitham et al., 1986). Agents that promote cell spreading, such as Fn (Werb and Aggeler, 1978), and agents that inhibit differentiation of some cell types, such as transforming growth factor- β (Edwards et al., 1987), inhibit the phenotypic expression of collagenase. On the other hand, substances that promote cell rounding, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), some proteinases, and cytochalasin B, induce expression of collagenase (Harris et al., 1975; Aggeler et al., 1984a,b; Werb et al., 1986; Unemori and Werb, 1986, 1988). Taken together, these data suggest that proteinases play a prominent role in regulating adhesion of cells to ECM.

Given the necessity for closely regulating the establishment and modulation of cell-ECM interactions, we used an adhesion-blocking monoclonal antibody against the Fn receptor (FnR) and Fn-derived peptides to explore the possibility that interference with normal cell-Fn interactions can alter gene expression. We show that ligation of the FnR by these ligands, but not by native Fn, induces the expression of metalloproteinases. In contrast to other agents that stimulate expression of these genes, the anti-FnR-mediated induction can occur in the absence of a major change in cell shape or in the reorganization of the actin cytoskeleton. These data suggest that the status of the Fn-FnR interaction is an important signaling mechanism in regulating the expression of genes relevant to matrix remodeling during differentiation.

Materials and Methods

Cells and Cell Culture

Rabbit synovial fibroblasts (RSF) isolated as described previously (Aggeler et al., 1984a) and used between passages 1 and 6 were cultured in DME supplemented with 10% FBS. Cells ($1-2 \times 10^5$) were plated in 16-mm wells for 14 h in this medium before washing and replacement with serum-free DME supplemented with 0.2% lactalbumin hydrolysate (DME-LH) for experiments. JAR human choriocarcinoma cells were cultured and maintained as described by Damsky et al. (1985a).

Preparation and Characterization of mAbs against Integrins

Whole JAR human choriocarcinoma cells were removed from the culture dishes with 5 mM EDTA, followed by washing with PBS containing Ca^{2+} and Mg^{2+} . A Lewis rat was immunized by a series of fortnightly injections of 10^7 JAR cells, twice intraperitoneally and twice intrasplenically. 4 d after the last injection the immune spleen cells were removed and fused with mouse Sp2/0 plasmacytoma cells and cultured according to the procedure of Kennett (1980) as modified by Wheelock et al. (1987). All wells with growing cells were screened by testing the ability of culture supernatants to inhibit the attachment of JAR cells to Fn or Ln in the attachment assay described by Giancotti et al. (1985) and Tomaselli et al. (1987). Two supernatants inhibited cell attachment to Fn only, whereas two others inhibited cell attachment to both Fn and Ln. After subcloning of the latter, subsequent characterization showed that the two mAbs also inhibited cell attachment to collagen types I and IV. None of the supernatants inhibited attachment to Vn. Detailed characterization of these mAbs will appear elsewhere (Hall, D. E., E. Crowley, and C. H. Damsky, manuscript submitted for publication). One mAb that inhibited cell attachment to Fn only (BIIG2) and one mAb that inhibited cell attachment to Fn, Ln, and the collagens (AIIB2) were selected for further study and designated anti-FnR and anti- β_1 , respectively. An unrelated cell-binding rat mAb, BIVF2, of the same subtype was used as a control. The second rat anti-FnR mAb (BIE5) was used in certain experiments where indicated.

The mAbs were further characterized by immunoprecipitation to determine which polypeptides on RSF were recognized by anti-FnR and anti- β_1 . Proteins in RSF ($\sim 5 \times 10^6$) were labeled by incubation for 24 h with 50 $\mu\text{Ci}/\text{ml}$ of [^3H]6-D-glucosamine (sp act 25 Ci/mmol; New England Nuclear, Boston, MA) in a low glucose (1 g/liter) formulation of DME. Cells were harvested in 2 mM EDTA, washed, and lysed in 10 mM Tris-acetate buffer, pH 8, containing 0.5% NP-40, 150 mM NaCl, and 2 mM PMSF. Precipitation of integrins by the anti-FnR and anti- β_1 mAbs was carried out according to the procedure described by Tomaselli et al. (1987). Immunoprecipitates were analyzed on 7% SDS-polyacrylamide gels under nonreducing conditions, followed by fluorography.

Preparation of Anti-FnR IgG and Monovalent Fab Fragments

IgG was purified from BIIG2 culture supernatant by anti-rat IgG affinity chromatography with goat anti-rat IgG immobilized on CNBr-activated agarose (Sigma Chemical Co., St. Louis, MO). The IgG was eluted with glycine, pH 2.6, neutralized immediately with Tris base, and dialyzed against PBS, pH 8.0. Affinity-purified BIIG2 in PBS, pH 8.0, was digested with papain (100 mg antibody per 1 mg papain) for 2 h at 37°C (Parham, 1986). The proteins were then alkylated with iodoacetamide (8 mM final concentration); the reaction mixture was dialyzed against 10 mM Tris-buffered saline, pH 8.0, or PBS, pH 8.0; and Fab fragments were separated from Fc fragments and undigested antibody by DEAE-cellulose or protein A-agarose chromatography. IgG and Fab concentrations are expressed in molar concentrations assuming molecular masses of 160 and 50 kD, respectively.

Preparation of ECM Ligand and Antibody Substrates

Culture dishes (Costar, Cambridge, MA; 24- or 48-well plates) were incubated with Fn or Vn at 10 $\mu\text{g}/\text{ml}$ in PBS, pH 7.4, overnight at 4°C. Unoccupied sites were blocked with 0.2% bovine serum albumin at ambient temperature for 2 h before cell plating. To assess the extent of collagenase expression on various substrates, cells were plated on Fn, Vn, anti-FnR IgG, or Fab. In some cases these substrates were covalently linked to 12-mm glass coverslips as described below.

Covalent Protein Coating of Glass Coverslips for Specific Adhesion

For covalently linked Fn, type I collagen, peptides containing the arg-gly-aspartate (RGD) cell recognition sequence, or purified anti-FnR IgG or Fab, polypeptides were conjugated to glass coverslips as follows. Coverslips were washed sequentially with 20% concentrated H_2SO_4 , water, 0.1 N NaOH, and water. Dried coverslips were exposed to γ -aminopropyltriethoxysilane (Sigma Chemical Co.) for 4 min at ambient temperature, followed by water and PBS rinses. The coverslips were incubated with 0.25% glutaraldehyde in PBS for 30 min at 22°C, washed several times with PBS, and covered

with ECM proteins or antibody solutions (20–700 µg/ml) for 1 h at ambient temperature, washed with PBS, and used immediately. All steps after the glutaraldehyde treatment were done with sterile reagents and utensils. Freshly trypsinized cells resuspended in DME-LH were plated on the glass coverslips containing immobilized proteins in 24-well plates.

Preparation of mAbs against Collagenase

Rabbit collagenase was purified from cultures of rabbit skin. Fragments of rabbit skin were cultured in DME-LH, and the cell-conditioned medium (CM) was collected every 2 d for up to 16–18 d. Medium from day 4 on was pooled and brought to 25% saturation with (NH₄)₂SO₄. The supernatant liquid was dialyzed extensively against 20 mM NH₄HCO₃, freeze-dried, reconstituted in water, and dialyzed against 10 mM Tris-HCl buffer, pH 8.4, with 0.05% Brij-35. Collagenase was purified by DEAE-Sephacel (Pharmacia Fine Chemicals, Piscataway, NJ) chromatography, followed by zinc-chelate affinity chromatography (Cawston and Tyler, 1979; Chin et al., 1985). The collagenase, which was eluted with 50 mM sodium acetate, pH 4.7, was purified 460-fold.

BALB/c mice were immunized with 100 µg of purified collagenase as described by Oi and Herzenberg (1980), except that Freund's complete adjuvant was used for the first injection and Freund's incomplete adjuvant was used for booster injections. The dispersed spleen cells of the mouse were fused with a subclone of the mouse myeloma line P3-X63-Ag8 that does not produce immunoglobulin. The fusion protocol was essentially the same as that described previously (Oi and Herzenberg, 1980), except that warm PBS instead of serum-free medium was added to the polyethylene glycol pellet, and the fused cells were centrifuged at 200 g for 8 min and resuspended in 12 ml of RPMI medium containing 15% FBS; 50 µl were placed in each well of a 24-well Costar plate for hybrid selection in medium containing 1×10^{-4} M hypoxanthine, 1×10^{-6} M aminopterin, and 1.6×10^{-5} M thymidine. As the hybrid cells grew, the medium was tested for production of specific antibody by a two-step, solid-phase ELISA, essentially as described by Maggio (1980), with alkaline phosphatase-conjugated anti-mouse IgG as secondary antibody. IgG secretors were subcloned and characterized by immunoblotting and immunoprecipitation of collagenase from TPA-treated RSF and by immunofluorescent staining of TPA-treated RSF. Relevant hybridoma clones were cultured in Ventrex HL-1 serum-free medium (Fisher Scientific Co., Pittsburgh, PA) or injected into BALB/c mice for ascites tumor production of antibody. mAb obtained from both sources was purified over a protein A-Sepharose column (Pharmacia Fine Chemicals). An "oligoclonal" mixture of five anticollagenase mAbs was used in this study.

Biosynthetic Labeling of Proteins Secreted by RSF

RSF proteins were biosynthetically labeled with 25–50 µCi/ml of [³⁵S]methionine (sp act 1,265 Ci/mmol) for 2–4 h in methionine-free DME at 37°C. Proteins were precipitated from the CM with quinine sulfate-SDS and resuspended in Laemmli sample buffer, as previously described (Unemori and Werb, 1986), and samples were analyzed on 10% gels with a 3% stacking gel under reducing conditions. Collagenase and stromelysin were immunoprecipitated from [³⁵S]methionine-labeled secreted proteins with 1–2 µg of monoclonal anti-rabbit collagenase IgG or 10 µg polyclonal sheep anti-stromelysin (Chin et al., 1985), followed by formalin-fixed *Staphylococcus aureus* (Zysorbin; Zymed Labs, Burlingame, CA). Nonimmune mouse IgG (2 µg) or sheep IgG (10 µg) replaced the immune IgG as controls. Total secreted proteins were separated on 7–15% or 10% SDS-polyacrylamide gels and then analyzed by fluorography as described previously (Unemori and Werb, 1986, 1988). All experiments were performed at least twice.

SDS-Substrate Gels for Analysis of Proteinases

CM was subjected to substrate gel electrophoresis in 10% polyacrylamide gels impregnated with 1 mg/ml gelatin or casein (Unemori and Werb, 1986; Herron et al., 1986). Unconcentrated CM was mixed with Laemmli sample buffer (lacking β-mercaptoethanol and modified to contain a final concentration of 2.5% SDS) and electrophoresed under nonreducing conditions. After electrophoresis, the gel was incubated in 2% Triton X-100 for 30 min at 37°C to remove SDS and then incubated for 18–24 h at 37°C in substrate buffer (50 mM Tris-HCl buffer, pH 8, containing 5 mM CaCl₂). After staining with Coomassie blue R250, gelatin- or casein-degrading enzymes present in the CM were identified as clear zones in a blue background.

RNA Isolation, Blotting, and Hybridization

Total RNA was isolated from cultured RSF by the method of either Chirg-

win et al. (1979) or Cathala et al. (1983). RNA was separated on agarose gels, transferred to nylon membranes, and probed with ³²P-labeled cDNA probes as previously described (Maniatis et al., 1982; Frisch et al., 1987). cDNA clones for rabbit collagenase (pCL1), stromelysin (pSL2; Frisch et al., 1987), human tissue inhibitor of metalloproteinases (TIMP) (SP65 TIMP/erythroid-potentiating activity; sequence identical to that described by Docherty et al., 1985; gift of M. Wrann, Sandoz Research Laboratories, Vienna, Austria), human 68-kD gelatinase/type IV collagenase (Collier et al., 1988; gift of G. Goldberg, Washington University, St. Louis, MO), and human γ-actin (Engel et al., 1981; gift of L. Keddes, Stanford University, Stanford, CA) were used to generate probes.

Colocalization of Collagenase and Actin Microfilament Bundles in RSF

Cells were plated in 24-well plates (Costar) in DME containing 10% FBS on 11-mm round glass coverslips for 8 h to permit spreading. Medium was then replaced with DME-LH. Anti-FnR IgG or control IgG was added at a concentration of 15 µg/ml. At various times, medium containing IgG was removed and replaced with DME-LH. At 24 h, coverslips were rinsed with DME-LH and fixed for 10 min in 3% paraformaldehyde in PBS containing 0.5 mM Ca²⁺ and then stained by a modification of the protocol of Damsky et al. (1985a). Briefly, after fixation, cells on coverslips were rinsed, made permeable with acetone at 4°C for 3 min, and incubated with PBS containing 0.2% bovine serum albumin. Cells were exposed for 1 h to a cocktail of mouse monoclonal antibodies against rabbit collagenase (Unemori and Werb, 1988). Cells were rinsed and exposed for 1 h to biotinylated goat anti-mouse IgG (Sigma Chemical Co.), rinsed again, and exposed for 30 min to a mixture of 1:100 dilution of FITC-labeled streptavidin (Amersham Corp., Arlington Heights, IL) and 1 µg/ml of rhodamine-phalloidin (Molecular Probes, Inc., Junction City, OR). The washed coverslips were then mounted in Gelvatol containing phenylenediamine to reduce quenching of the fluorescein signal (Platt and Michael, 1983) and examined with a phase epifluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with filters suitable for FITC and rhodamine and a 63× Planapo oil immersion objective. Cells were photographed with Eastman Kodak Co. (Rochester, NY) Tri-X film and developed with Accufine.

Other Antibodies and Reagents

A mouse anti-FnR mAb (gift of D. Cheresh, Scripps Clinic and Research Foundation, La Jolla, CA) was analyzed in certain experiments where indicated. A mouse mAb binding to the rabbit low density lipoprotein receptor (LDL-R) (gift of T. Innerarity, Gladstone Foundation Laboratories, University of California, San Francisco, CA) and a mouse mAb to the transferrin receptor (AMAC Inc., Westport, ME) were used as controls for antibodies binding to other membrane receptors. Gly-arg-gly-asp-ser-pro (GRGDSP) and gly-arg-gly-glu-ser-pro (GRGESP) peptides were gifts of E. Ruoslahti (La Jolla Cancer Research Foundation, La Jolla, CA) or were purchased from Telios, Inc. (La Jolla, CA). Gly-D-arg-gly-asp-ser-pro-ala-ser-ser-lys (GdRGDSPASSK), gly-arg-gly-asp-asn-pro (GRGDNP), and the 120-kD and 60-kD cell-binding fragments of Fn were purchased from Telios, Inc. Vn was a gift of Dr. M. Pierschbacher (La Jolla Cancer Research Foundation, La Jolla, CA); anti-Fn antibody, Ln, and Fn were purchased from Collaborative Research Inc., Waltham, MA. Type I collagen (Vitrogen) was purchased from Collagen Corp. (Palo Alto, CA).

Results

An mAb Recognizes the FnR in Rabbit Fibroblasts

We first tested an mAb to the FnR (BIIG2), one of the β₁ family of integrins, that was produced from a rat immunized with JAR human choriocarcinoma cells. Anti-FnR interfered with the initial attachment of many human cell types (data not shown), as well as RSF, to Fn (Fig. 1 A), but not to Ln, collagen types I and IV, or Vn (data not shown). Anti-FnR immunoprecipitated a dimer migrating at 110 and 150 kD under nonreducing conditions from NP-40 extracts of [³H]glucosamine-labeled RSF (Fig. 1 B). We then used another mAb, anti-β₁, which recognizes the common β-chain of the β₁-integrins, to determine which other members of this subfamily are present in RSF. Anti-β₁ interfered with

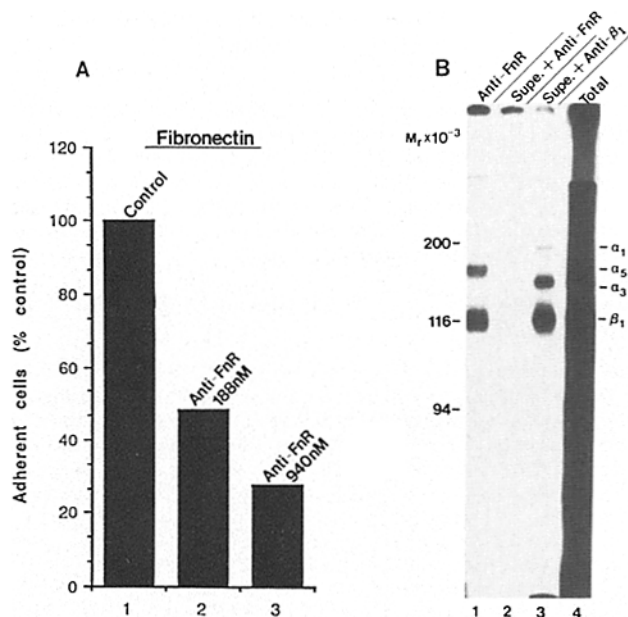


Figure 1. Characterization of an mAb that recognizes the FnR. (A) Inhibition of initial adhesion of RSF on culture wells coated with 10 $\mu\text{g/ml}$ of Fn by anti-FnR (BIIIG2) at 0.43 or 1.1 μM compared to the control mAb (BIVF2) at 0.63 μM . (B) Immunoprecipitation of integrins from NP-40 lysates of [^3H]glucosamine-labeled RSF. The immunoprecipitates were separated by SDS-PAGE under non-reducing conditions. The lysate was immunoprecipitated with anti-FnR (lane 1), and the supernatant (Supe) was immunoprecipitated sequentially with anti-FnR (lane 2) and anti- β_1 (lane 3); total lysate is shown in lane 4. The molecular weight standards ($\times 10^{-3}$) and the migration of the FnR α -chain (α_5), VLA₁ α -chain (α_1), VLA₃ α -chain (α_3), and β_1 -chain are indicated.

attachment of RSF and several human cell types to Fn, Ln, and collagen types I and IV, but not to Vn, a member of the β_3 -subfamily of integrins (data not shown). Anti- β_1 immunoprecipitated the 110-kD band of the β_1 -chain and two α -chains at 140 and 190 kD that are distinct from the FnR α -chain (Fig. 1 B). Sequential depletion experiments (data

not shown) indicated that these α -chains correspond to the integrins VLA₁ and VLA₃ (Takada et al., 1987). Thus, RSF express three members of the β_1 -family of integrins, including the FnR (VLA₅).

Anti-FnR Induces Expression of Collagenase and Stromelysin

Alteration of cell adhesion and shape by proteinases such as trypsin (Werb and Aggeler, 1978), culture on surfaces of varying adhesivity (Aggeler et al., 1984b), and collagen gel tension (Unemori and Werb, 1986) results in a change in gene expression in fibroblasts characterized by synthesis and secretion of the metalloproteinases collagenase and stromelysin. It was therefore of interest to determine whether perturbation of the FnR would affect collagenase and stromelysin gene expression. Accordingly, we cultured RSF for 24 h in uncoated tissue culture wells in medium containing serum and then treated them with anti-FnR or an unrelated rat mAb as a control. After treatment of RSF with anti-FnR, a striking induction of several secreted proteinases was observed by zymography of the CM in SDS-substrate gels containing gelatin (Fig. 2 A) or casein (Fig. 2 B). In particular, bands corresponding to the proenzymes of the metalloproteinases collagenase (Fig. 2 A) and stromelysin (Fig. 2 B) were visible. The induction of collagenase and stromelysin activity by anti-FnR was selective, because the expression of several other metalloproteinases, including the 68-kD gelatinase/type IV collagenase, was unchanged by the treatment.

Analysis of newly synthesized secreted proteins showed that polypeptides migrating at 51, 53, and 57 kD were induced in a concentration-dependent fashion by anti-FnR but not by control mAb (Fig. 3 A). mAbs recognizing two other membrane glycoprotein receptors, the LDL-R and the transferrin receptor, did not induce these polypeptides at concentrations of up to 625 nM (data not shown). Treatment of RSF with <25 nM (4 $\mu\text{g/ml}$) anti-FnR was effective in inducing expression of these polypeptides. Immunoprecipitation with specific antibodies indicated that the 53- and 57-kD bands were procollagenase and the 51-kD band was prostromelysin (Fig. 3 B). After treatment with anti-FnR, the two proen-

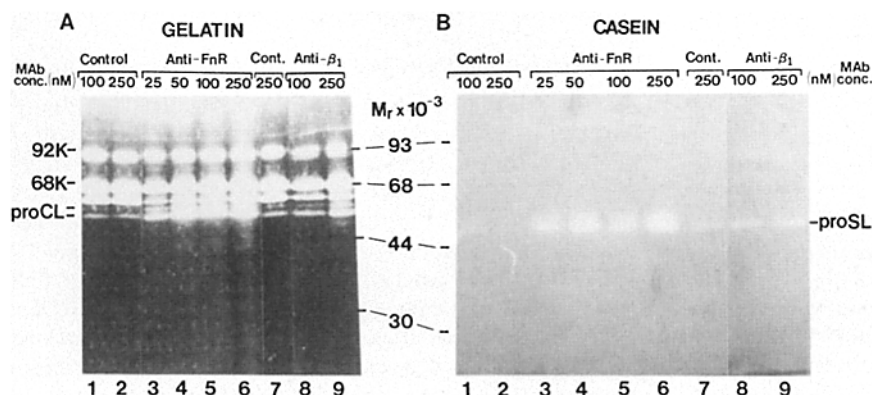


Figure 2. SDS-substrate gel zymography of secreted metalloproteinases induced by treating RSF with anti-FnR. RSF cultured in 48-well plates for 24 h in DME supplemented with 10% FBS were treated with various concentrations of control mAb (lanes 1, 2, and 7), anti-FnR (lanes 3-6), or anti- β_1 (lanes 8 and 9) as culture supernatants in DME-LH for 16 h. The cultures were then washed and incubated in DME-LH for 24 h. Samples (10 μl) of the CM were then separated on SDS-substrate gels containing either gelatin (A) or casein (B), and the zymograms were developed. The migration of prestained molecular weight standards and the bands corresponding to procollagenase (proCL), prostromelysin (proSL), 68-kD gelatinase (68K), and 92-kD gelatinase (92K) (Unemori and Werb, 1988) are indicated.

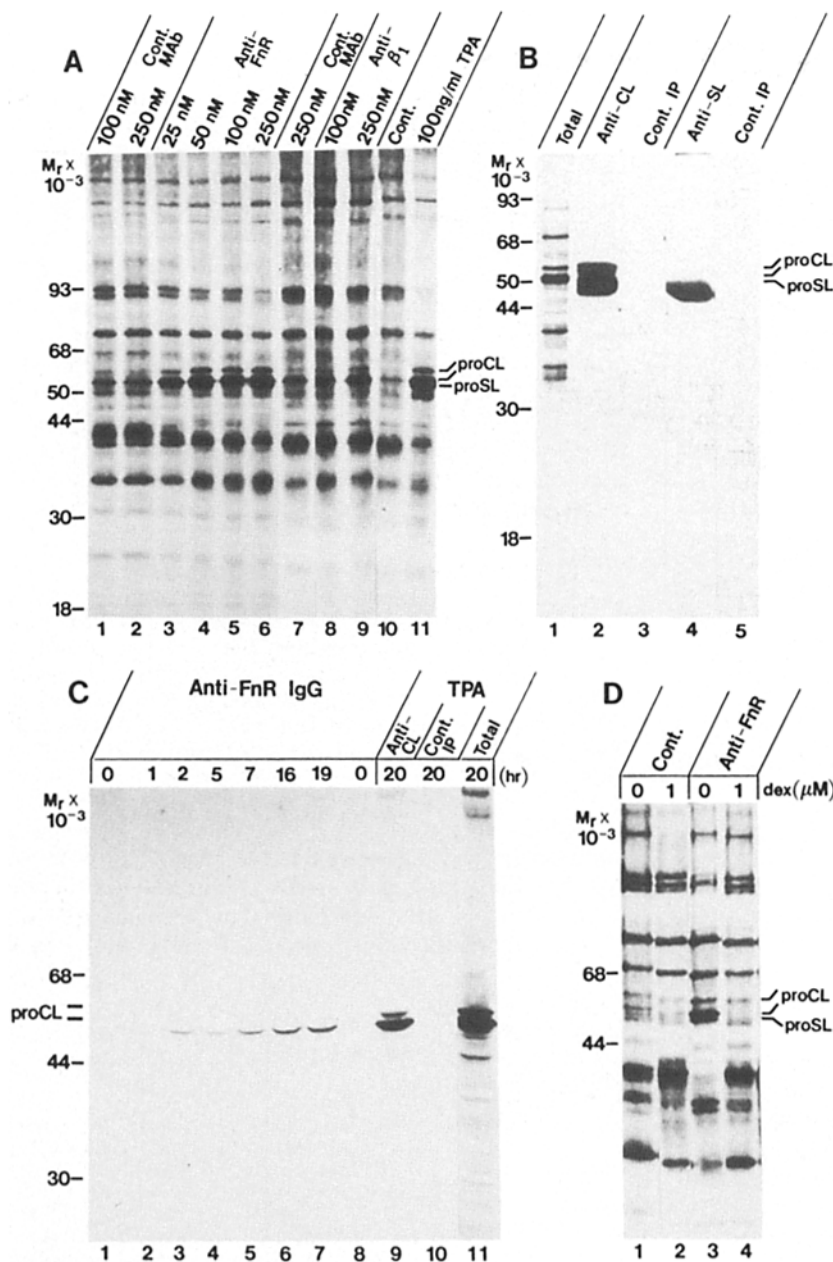


Figure 3. Expression of collagenase and stromelysin gene products by RSF treated with anti-FnR mAb. (A) RSF were treated with various concentrations of control mAb (lanes 1, 2, and 7), anti-FnR (lanes 3–6), or anti-β₁ (lanes 8 and 9) as culture supernatants, or TPA (lanes 10 and 11) as described in the legend to Fig. 2, then biosynthetically labeled with [³⁵S]methionine for 4 h. The secreted proteins were analyzed by SDS-PAGE followed by fluorography. (B) Collagenase and stromelysin bands were identified by immunoprecipitation of labeled secreted proteins from treated RSF. Total secreted proteins in 50 μl of CM are shown in lane 1. Proteins were immunoprecipitated from 400 μl of CM with anticollagenase IgG (lane 2), nonimmune mouse IgG (lane 3), antistromelysin IgG (lane 4), or nonimmune sheep IgG (lane 5), and analyzed by SDS-PAGE followed by fluorography. (C) Time course of induction of collagenase secretion by anti-FnR. Confluent RSF were incubated with 310 nM anti-FnR in DME-LH for 1–19 h and then anti-FnR was removed and the cells were cultured in DME-LH for the remaining time up to a total time of 20 h (lanes 1–8). The cells were then labeled with [³⁵S]methionine for 4 h. As a positive control, RSF were treated with TPA for 20 h (lane 9) before labeling. The CM containing the biosynthetically labeled proteins was collected, and collagenase was immunoprecipitated with anticollagenase and separated by SDS-PAGE. Lane 10 shows immunoprecipitation with control mouse mAb, and lane 11 shows the total secreted proteins of TPA-treated cells. (D) Effect of dexamethasone on induction of collagenase by anti-FnR. RSF were untreated (lane 1), treated with 1 μM dexamethasone (lane 2), or treated with anti-FnR as described in A, with (lane 4) or without (lane 3) 1 μM dexamethasone before biosynthetic labeling with [³⁵S]methionine and separation of secreted proteins by SDS-PAGE. Molecular weight standards (×10⁻³) and the migration of procollagenase (proCL) and prostromelysin (proSL) are indicated.

zymes accounted for as much as 5% of the total secreted proteins of RSF. Less than 2 h of exposure to anti-FnR was required for collagenase expression to be evident at the 24-h evaluation point (Fig. 3 C). Anti-β₁ also induced collagenase and stromelysin but was less effective than anti-FnR (Fig. 3 A). Two human fibroblast lines (MRC-5 and WI-38) also responded to treatment with the anti-FnR by induced expression of collagenase (data not shown). Two additional adhesion-blocking anti-FnR antibodies, the rat mAb (BIE5) and a mouse mAb, were qualitatively similar to the BIIG2 anti-FnR mAb in inducing collagenase and stromelysin expression in RSF (data not shown). In addition, dexamethasone suppressed the expression of the metalloproteinases induced by anti-FnR (Fig. 3 D). In the following sections we

concentrate on collagenase expression, although similar induction was generally seen for stromelysin.

We next used RNA blotting analysis to identify collagenase and stromelysin transcripts in RNA extracted from RSF treated with anti-FnR. Untreated RSF contained little mRNA for either metalloproteinase. Treatment of RSF with anti-FnR induced expression of mRNA for collagenase and stromelysin coordinately (Fig. 4) as in treatment with TPA (Frisch et al., 1987; Unemori and Werb, 1988) but in lower amounts (data not shown). In contrast, the expression of mRNA transcripts of the 68-kD gelatinase/type IV collagenase (Collier et al., 1988) and actin was constitutive and was not affected by treatment with anti-FnR (Fig. 4). The mRNA for TIMP decreased very slightly in response to anti-FnR, whereas it

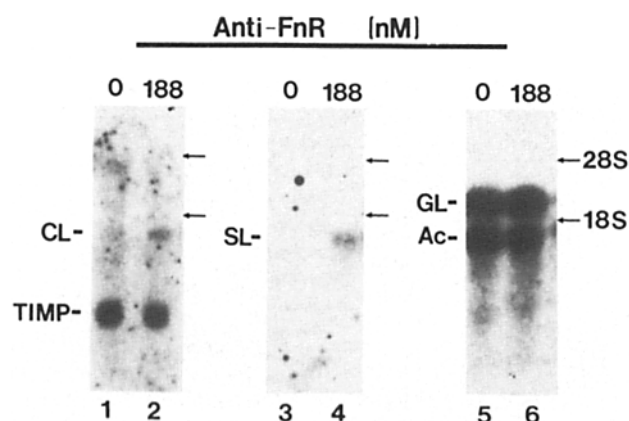


Figure 4. Regulation of metalloproteinase mRNA expression in RSF by anti-FnR. Blotting analysis of RNA isolated from untreated RSF (lanes 1, 3, and 5) or RSF treated with 188 nM anti-FnR (lanes 2, 4, and 6) for 14 h followed by 14 h in DME-LH alone. Total RNA (10 μ g) was separated on agarose gels, transferred to nylon membranes, and then hybridized with 32 P-labeled inserts from cDNAs encoding collagenase (CL) and TIMP (lanes 1 and 2), stromelysin (SL) (lanes 3 and 4), or 68-kD gelatinase (GL) and actin (Ac) (lanes 5 and 6). Migration of 28-S and 18-S rRNA are also indicated.

is induced by TPA (Murphy et al., 1985; Herron et al., 1986; Unemori and Werb, 1986).

Anti-FnR Induces Collagenase Expression in the Absence of Changes in Cell Shape

Previous experiments using TPA, cytochalasin D, calcium ionophore, collagen gel contraction, and poorly adhesive substrates indicated that induction of collagenase is strongly correlated with cell rounding and a substantial reorganization of the actin cytoskeleton, as determined by staining of actin filaments with rhodamine-phalloidin (Aggeler et al., 1984a,b; Unemori and Werb, 1986, 1988; Werb et al., 1986). When RSF were plated in the presence of serum that contained at least two adhesion proteins (Vn, Fn), they showed both a marked shape change (Fig. 5) and induction of collagenase expression (Fig. 6) after treatment with TPA. Under the same conditions, however, anti-FnR induced collagenase expression in the absence of apparent cytoskeletal reorganization: RSF maintained both a flattened morphology and elaborate arrays of rhodamine-phalloidin-staining actin microfilament bundles throughout the induction period (Fig. 5), and yet the cells went on to express collagenase (Fig. 6). These results are summarized in Table I. The adherent area of the anti-FnR mAb-treated cells differed from control RSF by <5% (data not shown).

Further evidence that shape change can be divorced from collagenase induction is indicated by the observations on cells plated and spread on immobilized anti-FnR as the substrate. Under these conditions, the cells displayed a flattened morphology but expressed collagenase (Table I; Fig. 7 A). Cells spread on type I collagen, Fn, or anti-LDL-R immobilized by the same procedure did not express collagenase. Therefore, immobilized anti-FnR acts as an inductive substrate, whereas several other substrates including Fn, the natural ligand of the FnR, do not.

To explore the relationship between cell shape and metal-

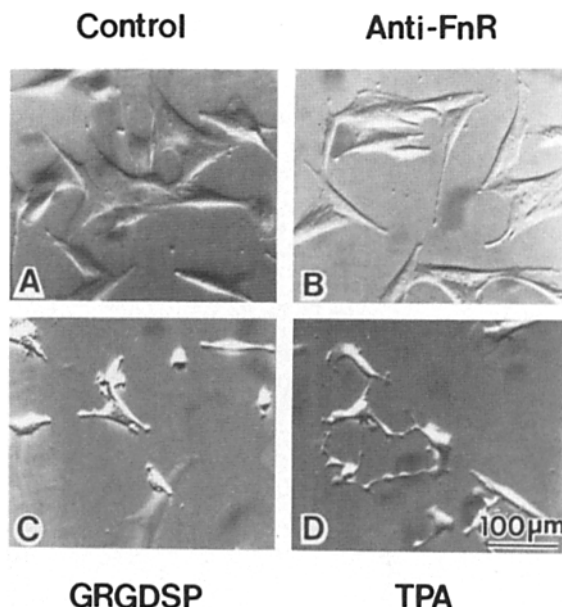


Figure 5. Low power morphological appearance of RSF treated with anti-FnR or a peptide containing RGD. RSF were treated with (A) control mAb (250 nM), (B) anti-FnR (250 nM), (C) GRGDSP (100 μ g/ml), or (D) TPA (50 ng/ml) for 24 h. Phase-contrast microscopy. Untreated RSF and RSF treated with GRGESP (not shown) were indistinguishable from RSF treated with control mAb in A.

loproteinase gene expression further, anti-FnR mAb was added to RSF cultured on covalently immobilized purified ECM substrates. Anti-FnR induced collagenase expression by RSF cultured on type I collagen, Fn (Fig. 7 A), and Vn

Table I. Lack of Correlation between Induction of Collagenase and Stromelysin Gene Expression and Changes in Cell Shape

Inducing agent	Cell substrate	Cell shape	Collagenase/stromelysin induced*
None	Serum	Flat	No
None	Collagen	Flat	No
None	Fn	Flat	No
None	Anti-LDL-R	Flat	No
TPA	Serum	Rounded	Yes
Cytochalasin†	Serum	Rounded	Yes
Collagen gel contraction†	Serum	Rounded	Yes
Proteinases†	Serum	Rounded	Yes
Soluble anti-FnR	Serum	Flat	Yes
Soluble anti-FnR	Fn	Rounded	Yes
Soluble anti-FnR	Collagen	Flat	Yes
Immobilized anti-FnR	Anti-FnR	Flat	Yes
GRGESP	Serum	Flat	No
GRGDSP	Serum	Rounded	Yes
Immobilized anti-FnR plus soluble GRGDSP	Anti-FnR	Rounded	Yes
GRGDSP	Collagen	Flat	No
Immobilized GRGDSP	GRGDSP	Flat	Yes
Immobilized Fn peptides	Fn peptides	Flat	Yes

* Induction of proteinases was determined by incorporation of [35 S]methionine into newly synthesized secreted proteins and/or by immunocytochemistry.

† Data are from Unemori and Werb (1986); Werb and Aggeler (1978); and Werb et al. (1986).

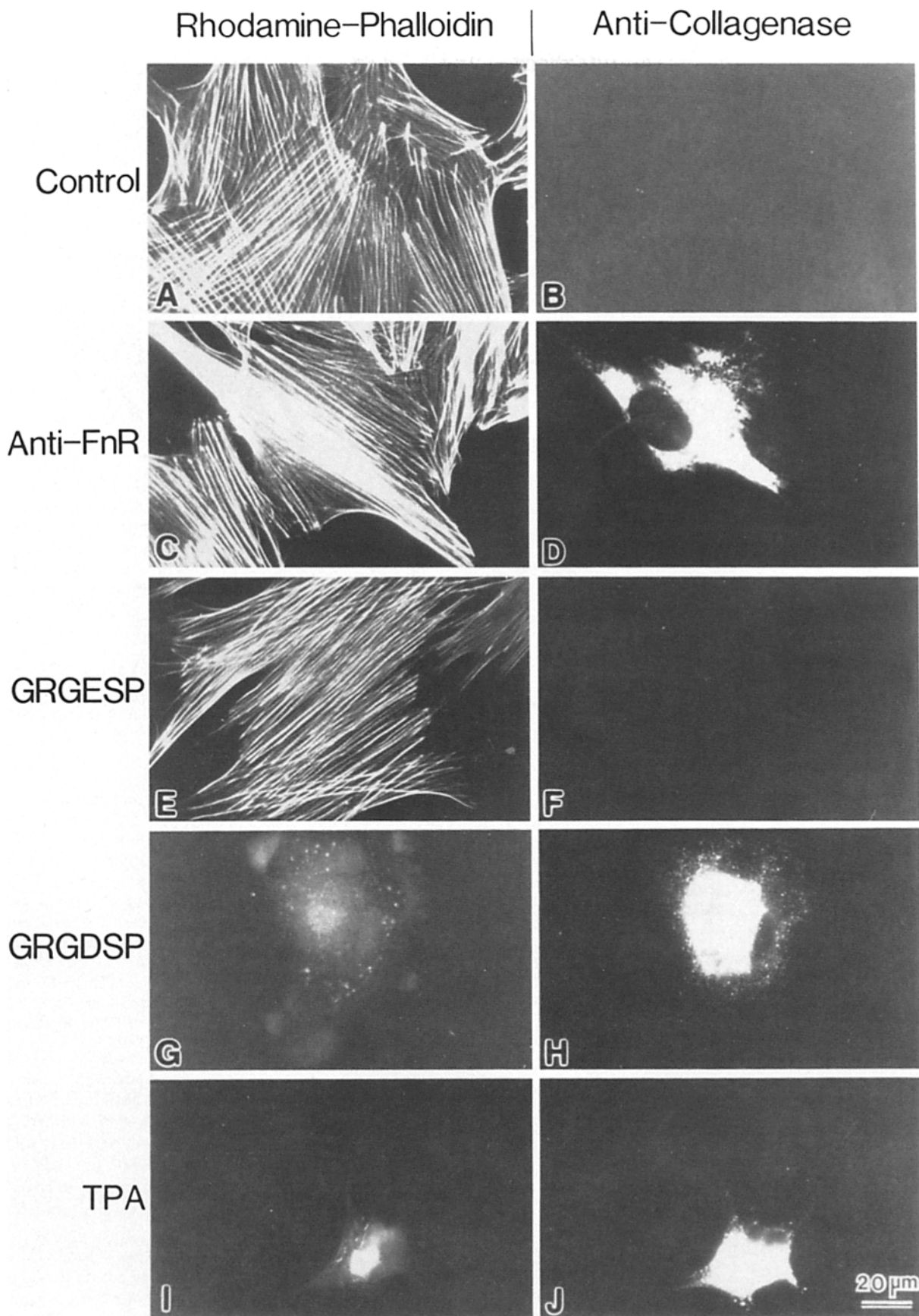


Figure 6. Localization of collagenase and actin microfilaments in RSF treated with anti-FnR or a peptide containing RGD. RSF plated on glass coverslips were left untreated (*A* and *B*) or treated with (*C* and *D*) anti-FnR (250 nM), (*E* and *F*) GRGESP (100 μ g/ml), (*G* and *H*) GRGDSP (100 μ g/ml), or (*I* and *J*) TPA (50 ng/ml), fixed, and double stained with (*left*) rhodamine-phalloidin to visualize actin microfilaments and (*right*) anticollagenase mAb followed by biotinylated anti-mouse IgG and fluorescein-streptavidin to visualize intracellular collagenase. Paired fluorescence micrographs are shown.

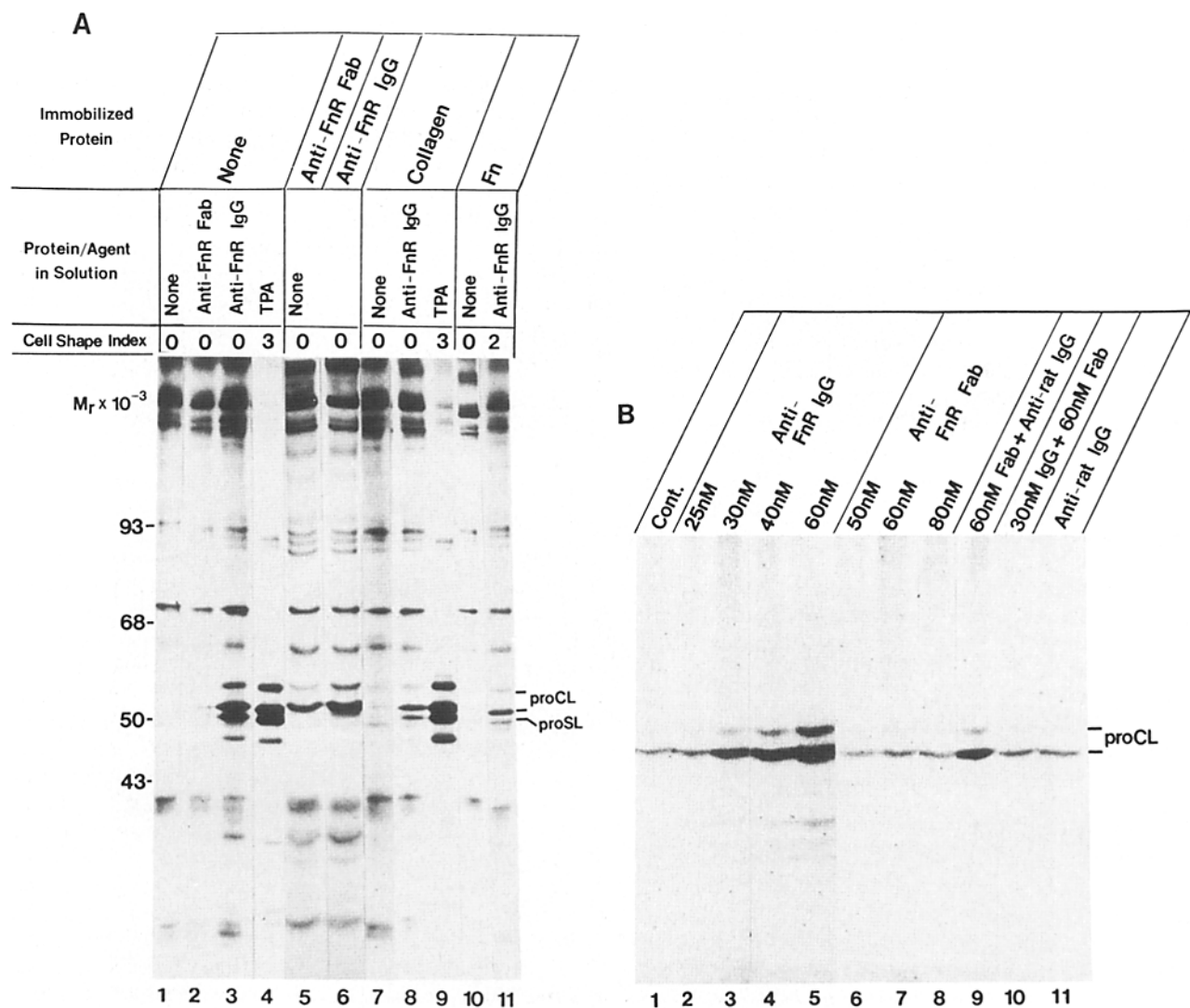


Figure 7. Induction of metalloproteinase expression by immobilized anti-FnR and requirement for cross-linked anti-FnR mAb. (A) RSF were plated on uncoated coverslips (lanes 1-4) in DME supplemented with 10% FBS for 4 h, then placed in DME-LH, or on coverslips coated with covalently immobilized anti-FnR Fab (lane 5), anti-FnR IgG (lane 6), type I collagen (lanes 7-9), or Fn (lanes 10 and 11) in DME-LH. Soluble anti-FnR Fab at 400 nM (lane 2), anti-FnR IgG at 115 nM (lanes 3, 8, and 11), or TPA at 100 ng/ml (lanes 4 and 9) was added. After 20 h the medium was removed and cultures were incubated with [³⁵S]methionine for 4 h to label proteins. The labeled secreted proteins were analyzed by SDS-PAGE followed by fluorography. The cell shape of the treated RSF, rated on a scale of 0-4, indicating flat to round but still attached (Aggeler et al., 1984b), is indicated across the top of the gel. The data are from two different experiments. (B) To examine the requirement for cross-linking, confluent RSF in 48-well plates were left untreated (lane 1) or treated with 25-60 nM anti-FnR IgG (lanes 2-5), 50-80 nM anti-FnR Fab (lanes 6-8), 60 nM anti-FnR Fab plus 80 μ g/ml rabbit anti-rat IgG (lane 9), or 30 nM anti-FnR IgG plus 60 nM anti-FnR Fab (lane 10). Control rabbit anti-rat IgG (80 μ g/ml) alone is shown in lane 11. The migration of procollagenase (proCL) is indicated.

(data not shown) but changed the cell shape only in cells cultured on Fn. Antibody to the Fn substrate itself also rounded up cells cultured on Fn and induced expression of collagenase in these cells (Table I).

Cross-linking Potentiates Induction of Collagenase Expression by Anti-FnR

The bivalent nature of antigen-antibody interactions suggests that cross-linking of the FnR in the plane of the membrane by anti-FnR may be important to the induction process. To evaluate this possibility, RSF were treated with monovalent anti-FnR Fab or bivalent anti-FnR IgG. Even at twice the equimolar concentration, monovalent anti-FnR

Fab was much less effective than bivalent anti-FnR IgG in inducing expression of metalloproteinase, and competing anti-FnR Fab reduced the inducing effect of anti-FnR IgG (Fig. 7 B). Inducing activity of the anti-FnR Fab was increased when a secondary anti-rat IgG was added to cross-link the FnR artificially by interacting in a bivalent manner with the anti-FnR Fab.

A second line of evidence that cross-linking of the FnR by anti-FnR is important comes from the observation that immobilization of monovalent anti-FnR Fab on the substrate enhanced its collagenase-inducing activity (Fig. 7 A). These data suggest that aggregation of the FnR by anti-FnR, and not just occupancy by the anti-FnR mAb, is important in the gene induction events (Table I).

Fibronectin-derived Peptides Induce Collagenase and Stromelysin Expression

Although antibodies to receptors may behave as high affinity ligands, the anti-FnR mAb did not mimic the effects of native Fn. Therefore, we sought another physiological ligand with the same effector functions as the antibody. Hexapeptides containing RGD, which is present in Fn, Vn, and other ECM ligands that interact with integrins, interfere with cell adhesion (Pytela et al., 1986; Pierschbacher and Ruoslahti, 1987) and differentiation events (Menko and Boettiger, 1987). If the effects of anti-FnR on metalloproteinase gene expression are due to interference with the interaction of RSF with Fn, then RGD peptides should also induce metalloproteinases. We found that the GRGDSP peptide, but not the control GRGESP peptide, when added to RSF cultured in the presence of serum, induced a concentration-dependent increase in collagenase and stromelysin synthesis and secretion, as analyzed by zymography (Fig. 8 A) and by biosynthetic labeling of newly synthesized secreted proteins (Fig. 8 B). However, unlike the treatment with anti-FnR mAb, treatment with the GRGDSP peptide caused a marked shape change and reorganization of actin microfilament bundles along with the induction of collagenase expression (Figs. 5, 6, and 8, A and B). The GRGESP peptide produced no shape change, actin rearrangement, or collagenase induction.

Because the GRGDSP sequence is not recognized by the collagen receptor of the integrin class, binding to collagen is not reversed by this peptide (Dedhar et al., 1987). In contrast to cells plated in serum, when the GRGDSP peptide was added to RSF plated on a collagen substrate the cells did not round up, and metalloproteinase gene expression was not induced (Table I). These results were surprising because they suggested that the RGD peptide induces metalloproteinase gene expression by a mechanism dependent on a change in cytoarchitecture rather than the shape-independent mechanism induced by the anti-FnR. On the other hand, the GRGDSP peptide caused cell rounding in the RSF plated on immobilized anti-FnR (Table I), suggesting that anti-FnR recognizes a site on the FnR close to that bound by the RGD cell-recognition sequence on Fn.

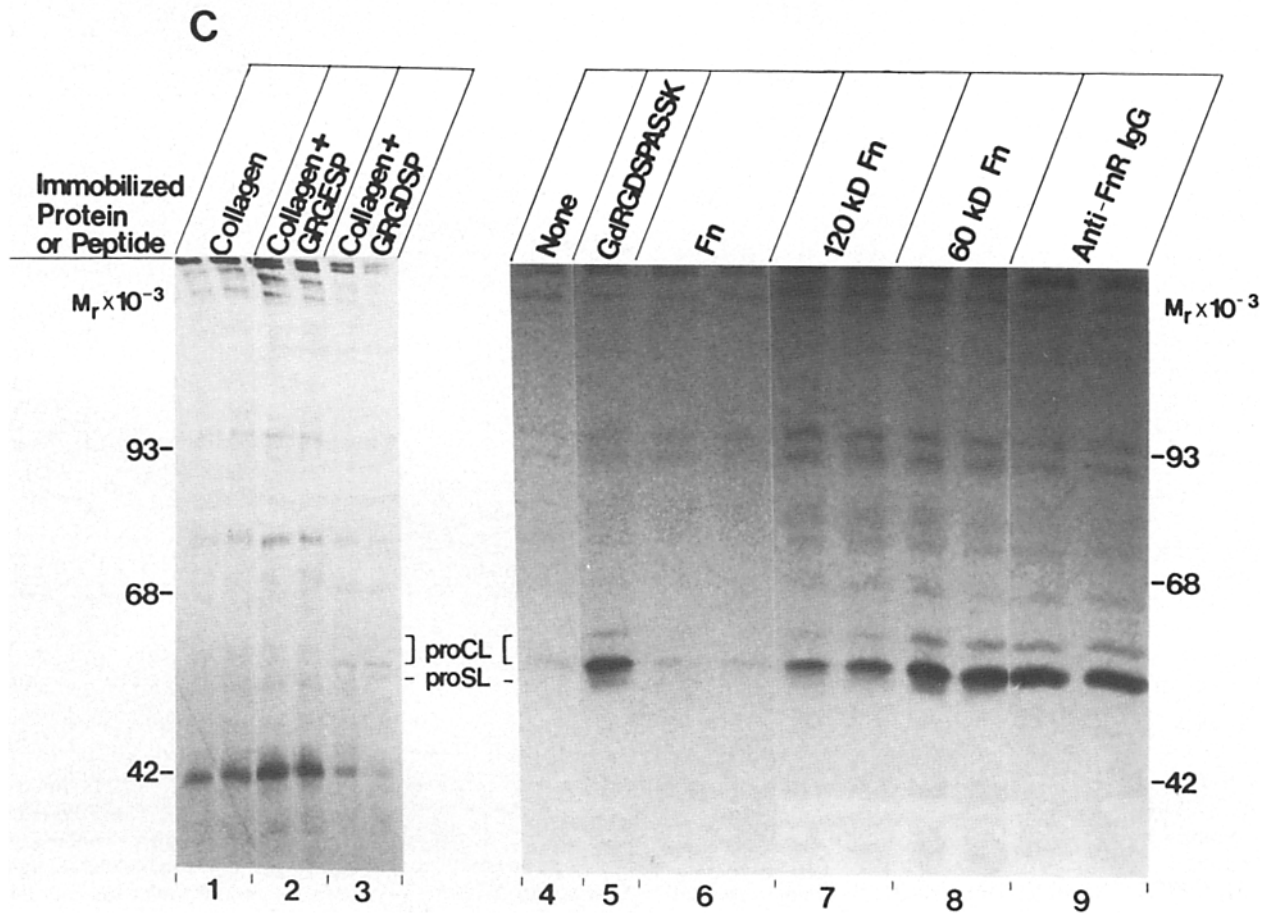
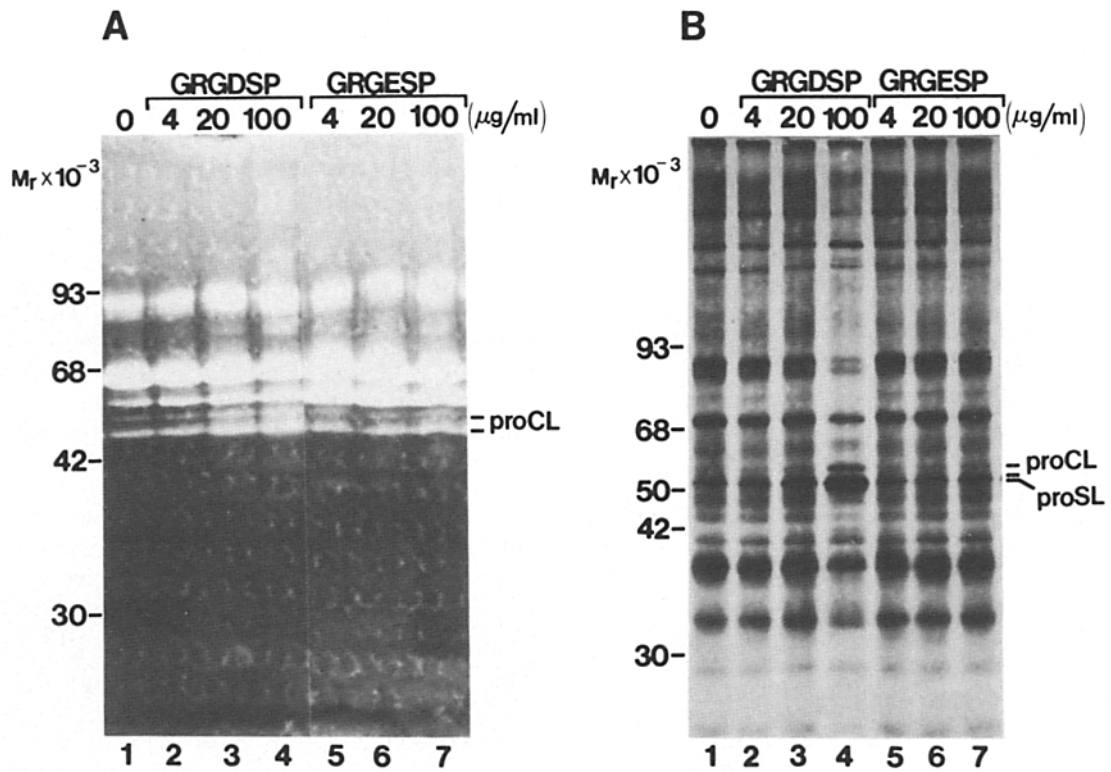
These disparate observations can be reconciled with the results with the anti-FnR mAb when we consider that the RGD peptide is a monovalent ligand. If the RGD peptide behaves as the natural inductive ligand for the FnR receptor only when it is present in a multimeric form, then immobilization of the peptide on the substrate should potentiate its inductive effect. RSF spread on immobilized GRGDSP, but not on GRGESP, rendering GRGESP inappropriate as a control. However, when GRGDSP or GRGESP was immobilized by cross-linking in the presence of collagen, RSF spread and assembled an organized actin cytoskeleton on both substrates. Collagenase expression was induced only in the cells spread on the immobilized GRGDSP, not on GRGESP or collagen alone (Fig. 8 C). Soluble, monovalent GRGDSP had no effect on RSF plated on collagen (Table I). Because GRGDSP is recognized not only by the FnR but also by the Vn receptor and other integrins, it has less selectivity than does the anti-FnR mAb. Therefore, we tested two other peptides, GdRGDSPASSK and GRGDNP, which have a higher specificity for the FnR (Pierschbacher and Ruoslahti, 1987); these peptides were also effective in inducing collagenase synthesis when immobilized.

We then tested whether larger fragments of Fn that contain the cell-binding domain and RGD sequence can mimic the inductive effects of the anti-FnR mAb. Collagenase and stromelysin expression was induced when RSF spread on immobilized 60- and 120-kD Fn fragments, in contrast to the lack of expression on immobilized native Fn (Fig. 8 C; Table I). The induction of metalloproteinases was dependent on the concentration of the immobilized Fn-derived peptides in the presence of collagen (data not shown). Although collagenase expression was also induced when the Fn fragments were added in solution (data not shown), we could not verify their monovalent status because of problems with aggregation of the fragments or their sticking to the surface of the culture dishes. Taken together, these data suggest that binding of Fn-derived peptides to the FnR triggers events different from those triggered by binding of native Fn.

Discussion

Our data demonstrate that perturbation of the interaction of Fn with its specific heterodimeric integrin receptor can alter gene expression. Treatment of attached and spread fibroblasts with an mAb against the specific integrin heterodimeric FnR, or with peptides containing the RGD cell-recognition sequence of Fn, but not with native Fn, induced expression of the genes for the metalloproteinases collagenase and stromelysin. Phorbol diesters, cytochalasins B and D, growth factors, and poorly adhesive substrates have also been shown to induce expression of these genes in fibroblasts (Aggeler et al., 1984a,b; Unemori and Werb, 1986, 1988; Edwards et al., 1987; Frisch and Ruley, 1987; Werb, 1989). The induction of proteinase expression by triggering the FnR and by these other treatments is similar in at least two respects. In all cases, there is a lag period before increased enzyme secretion is detectable. Once the inducing agent has been present for several hours, it can then be removed and the cells will go on to produce metalloproteinases over the next 24–48 h. Furthermore, proteinase induction by anti-FnR, as well as by the other inducers (Frisch and Ruley, 1987; Werb, 1989), is inhibitable by dexamethasone.

Despite these similarities, there is at least one important distinguishing feature between induction of proteinase expression mediated by the FnR and induction by previously reported treatments. Fibroblasts are induced to express metalloproteinases by anti-FnR even though they can remain flat and well spread throughout the induction period. Other treatments induce significant shape changes, which correlate with the extent of collagenase induction (Aggeler et al., 1984b; Werb et al., 1986). The divorcing of shape change from proteinase induction of collagenase and stromelysin gene expression by FnR has been documented in two ways. First, the organization of microfilament bundles, as detected by rhodamine-phalloidin, is not substantially altered during exposure to anti-FnR, whereas other agents, including the peptide containing RGD added in solution to cells spread in the presence of the adhesion proteins in serum, cause persistent and pronounced cell rounding and/or cytoskeletal reorganization. Although we cannot rule out the possibility that altered ligation of the FnR by the anti-FnR in solution induces more subtle or transient changes in cytoarchitecture, such as those seen in the first few minutes after the administration of growth factors such as epidermal growth factor



(Chinkers et al., 1979), in previous studies induction of proteinases was correlated with marked generalized alterations of the cytoskeleton of several hours' duration (Werb et al., 1986). In addition, studies on endothelial cells have shown that the FnR and Vn receptors are organized independently by their ligands, but both lead to local assembly of focal contacts and cytoskeletal proteins (Dejana et al., 1988). Thus, in cells exposed to serum, the cytoarchitecture may be maintained by the Vn receptor in the face of disruption of the FnR-Fn interaction. Second, proteinase expression by fibroblasts is stimulated even if they are cultured on substrates of anti-FnR, or of Fn-derived peptides containing RGD, that have been covalently linked to the culture dish. In this case, the fibroblasts spread on the mAb and the other immobilized inductive ligands as well as they do on noninductive collagen and Fn substrates and assume a highly flattened morphology, but are able to express proteinases. When Fn is conjugated to the dish, the cells spread but do not express proteinases. Thus, cells can distinguish whether they are attached to the substrate via their natural intact ligand or via Fn subfragments and the epitope recognized by the anti-FnR mAb, even though in all cases the cells have a similar morphology and degree of spreading. Because the selection screens for the anti-FnR mAb depended on inhibition of adhesion of cells to Fn, and peptides containing RGD also inhibit adhesion, it is likely that the anti-FnR mAb is a high affinity ligand for the subset of FnR configurations recognized by Fn subfragments. There are few data in the literature suggesting that Fn and Fn-derived peptides are recognized differently. However, monocytes, a cell type that expresses the FnR, show chemotaxis toward the cell-binding fragment of Fn but not native Fn in solution (Clark et al., 1988). Interestingly, these cells will respond to GRGDSP by activating their complement receptors only when bound to a surface (Wright and Meyer, 1985). Taken together, these observations suggest that the conformation of the binding site in Fn recognized by the FnR is likely to be altered when Fn is degraded by enzymes such as stromelysin (Chin et al., 1985).

There are other systems, such as adipocyte (Spiegelman and Ginty, 1983) and chondrocyte (Zanetti and Solursh, 1984) differentiation, in which the strong relationship between changes in cell shape and the induction of gene expression has also been observed. In these experiments, ECM molecules that reverse or prevent the shape change, such as Fn, prevent the induction of new gene expression. It is plausible that during induction of adipocyte-specific genes in preadipocytes and cartilage-specific genes in chondroblasts, as well as during induction of metalloproteinases in RSF by

shape-altering reagents, the correlated reorganization of the actin cytoskeleton may act indirectly to alter adhesion of the cells via the FnR or other integrins. Therefore, these inductive signals may actually be transduced by the integrins.

Although RSF respond to anti-FnR whether it is presented in solution or substrate-bound, the two phenomena are fundamentally different in several respects and could be regulated by separate signaling mechanisms. In the first case, RSF are presented with soluble mAb after they have spread on the multiple adhesion proteins from serum and have formed a stable cytoskeletal framework. The effect of the soluble mAb would then be expected to be directly inductive for gene expression. In contrast, when freshly trypsinized, rounded RSF, with a disorganized cytoskeleton and diffuse FnR distribution, are plated on the immobilized anti-FnR, it is the interaction with mAb, not the native adhesion ligands, that induces the spreading of the cells. Because cell rounding and actin disorganization are correlated with metalloproteinase expression, it is possible that, upon exposure of cells to the native ligand, the inhibitory signal usually generated by interaction of Fn and other adhesion proteins with integrins stops the induction of metalloproteinases. However, when RSF are spreading on anti-FnR or Fn-derived peptides, the inhibitory signal does not go into effect, and gene expression is induced by a default pathway. These mechanisms remain to be explored.

Our data showing that anti-FnR and peptides containing RGD are inductive for metalloproteinase gene expression when multimeric or immobilized, but not when in solution in monovalent form, suggest that aggregation of the receptor is a prerequisite for transfer of information. The effects are specific to the FnR; cross-linking of other glycoprotein receptors such as the LDL-R and transferrin receptor did not induce the metalloproteinases. Although the distribution of the FnR on RSF was not determined directly in the present study, because this anti-FnR mAb does not stain well, there is a correlation between receptor aggregation and receptor function for two other integrins, Mac-1 of the β_2 -subclass and GPIIb/IIIa of the β_3 -subclass (Detmers et al., 1987; Isenberg et al., 1987). Therefore, cross-linking of the FnR may constitute part of the mechanism for transducing the signal for collagenase induction as it does for a variety of other receptors, including those for insulin (Kahn et al., 1978) and epidermal growth factor (Wakshull and Wharton, 1985). It is of interest in this regard that a fibrinogen decapeptide is able to induce aggregation of GPIIb/IIIa in platelets. However, aggregation alone clearly does not trigger the biological response, because the FnR is aggregated in focal contacts

Figure 8. Induction of metalloproteinase expression by peptides containing an RGD sequence. (A and B) Monolayers of RSF cultured to confluence in medium containing serum in 48-well plates were left untreated (lane 1) or incubated with various concentrations of the GRGDSP peptide (lanes 2–4), which contains the cell adhesion recognition sequence for integrins, or with the control GRGESPP peptide (lanes 5–7) in DME-LH for 24 h. The cell shape indices of the treated RSF in lanes 1–7 were 0, 1, 2, 3, 0, 0, and 0, respectively. (A) Samples (10 μ l) of the CM were then analyzed for secreted proteinases by zymography on an SDS-gelatin substrate gel. (B) The cells were then biosynthetically labeled with [35 S]methionine for 4 h and the secreted proteins were analyzed by SDS-PAGE followed by fluorography. (C) RSF were plated on coverslips coated with immobilized proteins or peptides prepared by covalently cross-linking type I collagen at 1 mg/ml (lane 1), collagen at 1 mg/ml plus GRGESPP at 40 μ g/ml (lane 2), collagen at 1 mg/ml plus GRGDSP at 40 μ g/ml (lane 3), GdRGDSPASSK at 200 μ g/ml (lane 5), native Fn at 10 μ g/ml (lane 6), 120-kD fragment of Fn at 10 μ g/ml (lane 7), 60-kD fragment of Fn at 10 μ g/ml (lane 8), or anti-FnR mAb at 140 μ g/ml (lane 9). Lane 4, RSF plated on plain glass coverslips. Some of the samples are shown in duplicate. After incubation for 30 h (lanes 1–3) or 34 h (lanes 4–9) in DME-LH, the cells were biosynthetically labeled with [35 S]methionine for 4 h and the secreted proteins were analyzed by SDS-PAGE followed by fluorography. Molecular weight standards ($\times 10^{-3}$) and the migration of procollagenase (*proCL*) and prostromelysin (*proSL*) are indicated.

when cells adhere to Fn, but no collagenase is induced under those conditions. Thus, it is likely that the inductive ligands produce a change in receptor conformation and/or interaction with the membrane along with oligomerization of the receptor and that this change contributes to signal transduction. The anti-FnR mAb and Fn-derived peptides induce a different subset of signals and responses than does the natural ligand Fn. In platelets, it is possible that different ligands induce different conformations of GPIIb/IIIa (Phillips et al., 1988). Taken together, all these results point to the conclusion that the nature of the interaction of the FnR with its ligand is an important factor in regulating gene expression. Thus, in addition to mediating cell attachment and spreading on Fn and formation of a membrane-actin cytoskeleton complex, the FnR complex can mediate signal transduction between the external environment and the cell interior. The nature of the signaling molecules is currently under investigation.

The integrins are good candidates to be involved in signal transduction. Integrins are transmembrane heterodimers that interact directly with their ECM ligands (Horwitz et al., 1985; Pytela et al., 1986; Tomaselli et al., 1988; Gehlsen et al., 1988b; Gailit and Ruoslahti, 1988) and with talin, a molecule associated with the cytoskeleton (Burridge, 1986; Buck et al., 1986). Tyrosine phosphorylation on the β -chain may regulate affinity for both Fn and talin (Burridge, 1986; Hirst et al., 1986; Buck and Horwitz, 1987). Our data suggest that integrins can act as a kind of homeostatic system for modulating ECM structure and organization in response to the needs of the cell. The high affinity interactions of mAbs with integrins mimic the interactions of integrins with fragments of ECM ligands, and thus make them useful for studying the pleiotropic ligands and functions of these receptors.

ECM remodeling is particularly important during embryonic development, in wound healing, in chronic inflammation, and in metastasis and embryo implantation (Fairbairn et al., 1985; Mignatti et al., 1986; Schultz et al., 1988; Sutherland et al., 1988). As a result of wounding, for example, there are likely to be significant changes in the environment of the cells at the wound site, including generation of fragments of ECM ligands, with an ordered series of events requiring ECM degradation, cell migration, ECM resynthesis, and remodeling. The pleiotropic responses of cells to interaction of their integrins with a changing population of ligands may therefore not only reflect the changes in the cellular environment but may actively mediate them through changes in expression of ECM and ECM-degrading molecules.

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